Synthesis, Crystal Structure, and Enhancement of the Efficacy of Metronidazole Against *Entamoeba histolytica* by Complexation with Palladium(II), Platinum(II), or Copper(II)

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Reaction of *trans*-$[PdCl_2(DMSO)_2]$, *cis*-$[PtCl_2(DMSO)_2]$, and $[Cu(OAc)_2]\cdot H_2O$ with metronidazole (mnz) leads to the formation of new complexes, *i.e.*, *trans*-$[PdCl_2(mnz)_2]$ (**1**), *trans*-$[PtCl_2(mnz)_2]$ (**2**), and *trans*-$[Cu_2(OAc)_4(mnz)_2]$ (**3**), respectively. Complexes **1**–**3** crystallize all in the centrosymmetric monoclinic space group $P2_1/c$ with $Z=8$. Unit-cell parameters for these complexes are: **1**, $a=7.1328(14)$ Å, $b=20.699(4)$ Å, $c=7.1455(14)$ Å, and $\beta=116.17(3)^\circ$; **2**, $a=6.9169(14)$ Å, $b=21.853(4)$ Å, $c=6.7218(13)$ Å, and $\beta=110.79(3)^\circ$; **3**, $a=9.1663(18)$ Å, $b=19.129(4)$ Å, $c=8.9446(18)$ Å, and $\beta=116.44(3)^\circ$. The complexes **1** and **2** maintain an ideal square-planar geometry. In complex **3**, the H_2O molecules of the starting complex are replaced by metronidazole while maintaining a dimeric structure of $[Cu(OAc)_2]$. Each Cu ion has an ideal octahedral structure, though distortion occurs in the equatorial position where the acetato ligands are attached. The Cu–Cu separation of 2.6343(8) Å indicates considerable metal-metal interaction. The testing of the antiamoebic activity of these complexes against the protozoan parasite *Entamoeba histolytica* suggests that compound **1**–**3** might be endowed with important antiamoebic properties since they showed IC_{50} values in a μM range better than metronidazole (*Table 2*). Thus, compound **1** displayed more effective amoebicidal activity than metronidazole (IC_{50} values of 0.103 μM *vs.* 1.50 μM, resp.).

Introduction. – *Entamoeba histolytica* (*E. histolytica*), the cause of amoebic dysentery and amoebic liver abscesses, remains a significant threat to health in large parts of the world. More than 50 million people are affected, and only malaria and schistosomiasis surpass amoebiasis as a parasitic cause of death [1]. It is responsible for 100000 deaths annually [2]. The morbidity and mortality associated with amoebiasis have persisted despite the availability of effective antiamoebic therapy, suggesting a need for alternative measures of disease control. Nitroimidazole drugs such as metronidazole are highly effective against the acute disease but are relatively ineffective in eradicating parasites from the gut lumen [3]. Moreover, tolerance of these drugs is often poor and the mutagenic effects of metronidazole in bacteria have raised fear that the drug may be carcinogenic in man [4], although no evidence of carcinogenicity was found when exploratory studies were carried out [5][6]. To date, there have been a few reports of resistance of *E. histolytica* to metronidazole, but the possibility of this developing must be borne in mind [7], especially as emetine-resistant mutants have been isolated in the laboratory [8]. Metal ions are known to accelerate

drug action, and the efficacy of a therapeutic agent may be enhanced upon coordination with a metal ion [9]. Many neutral palladium(II) and palladium(IV) complexes were found to exhibit potential antitumor activity [10][11]. Copper(II) is a biologically active essential ion; its chelating ability and positive redox potential allow its participation in biological transport [12]. Some metal complexes have been proved to be active against trypanosoma [13–15], leishmania [14], and malaria [16]; their potential as antiamoebic agents has so far been little explored [17–19].

In this paper, we present a novel approach towards the development of chemotherapy against amoebiasis, consisting of the modification of metronidazole (=2-methyl-5-nitro-1*H*-imidazole-1-ethanol; mnz) by coordination with Pd^{II}, Pt^{II}, and Cu^{II}. These new complexes are active against *E. histolytica in vitro* and *in vivo*. This study establishes the potential for developing new antiamoebic drugs that are more effective than mnz and which may prove to be useful clinical alternatives to mnz.

Results and Discussion. – *Synthesis and Characterization of the Complexes.* Reaction of mnz with *trans*-$[PdCl_2(DMSO)_2]$ [20], *cis*-$[PtCl_2(DMSO)_2]$ [20], and $[Cu(OAc)_2]\cdot H_2O$ in refluxing MeOH resulted in the formation of *trans*-$[PdCl_2(mnz)_2]$ (**1**), *trans*-$[PtCl_2(mnz)_2]$ (**2**), and *trans*-$[Cu_2(OAc)_4(mnz)_2]$ (**3**), respectively. Whether the starting material was *cis*- or *trans*-configured, the resulting complexes were *trans* only (*vide infra*) under the reaction conditions. These complexes were purified by recrystallization. All complexes **1**–**3** are stable in air, soluble in coordinating solvents such as MeOH, EtOH, DMSO, and DMF, and insoluble in noncoordinating solvents like C_6H_6, $CHCl_3$, and CH_2Cl_2. Besides elemental analysis, all the new complexes were well-characterized by UV/VIS, IR, and ^{1}H-NMR spectroscopy and by crystal-structure analysis. Thermogravimetric analysis of the complexes showed them to be very stable and to decompose above 200° in two or three steps. These decomposition steps overlapped, and it was impossible to judge exactly which group was lost. The IR spectra of the complexes exhibit a sharp band at 1558–1575 cm^{-1} due to $\tilde{\nu}$(C=N) (ring); a positive shift $\Delta\tilde{\nu} = 21–38$ cm^{-1} with respect to the corresponding absorption of the free ligand indicates the coordination of the ring N-atom to the metal ion. The alcoholic group remains free and does not participate in bond-formation as $\tilde{\nu}$(C–O) (alcohol) appears at a nearly constant position of 1187 cm^{-1} in both mnz and its complexes. To determine the coordination geometry of the Pd and Pt complexes, far-IR studies were undertaken; thus, both complexes **1** and **2** exhibit a single M–Cl stretch at 374 and 361 cm^{-1}, respectively. The appearance of a single IR-active band indicates that the two Cl^- ligands of **1** and **2** are *trans* to each other. In addition to this, all complexes exhibit a broad band due to $\tilde{\nu}$ (OH) at *ca.* 3400 cm^{-1}. This is further supported by the ^{1}H-NMR data of **1**–**3** (*t* at δ *ca.* 6.0 due to free OH; moreover δ 4.36–4.49 (*t*) and 3.56–3.71 (*q*) for CH_2Cl_2). The structures of complexes **1**–**3** were confirmed by X-ray-diffraction studies.

Crystal Structures of Complexes **1**–**3**. The Pd and Pt complexes **1** and **2** crystallized from ^{i}PrOH/MeOH and the Cu complex **3** from dimethylsulfoxide (DMSO)/MeOH. The molecular structures and atomic numbering for **1**–**3** are shown in *Figs. 1–3*. Selected bond distances and angles for all complexes are summarized in *Table 1*. Complexes **1** and **2** are isostructural, exhibiting similar unit-cell volumes and crystallizing in the same space group. Moreover, an overlap plot of the two molecules

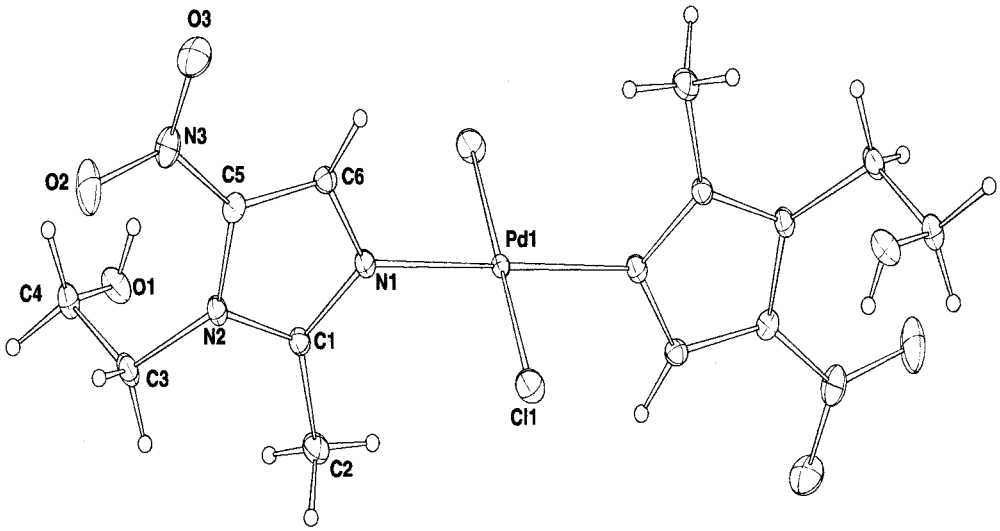

Fig. 1. *Molecular structure and atomic numbering* (arbitrary) *for complex* **1**. Ellipsoids at 30% probability.

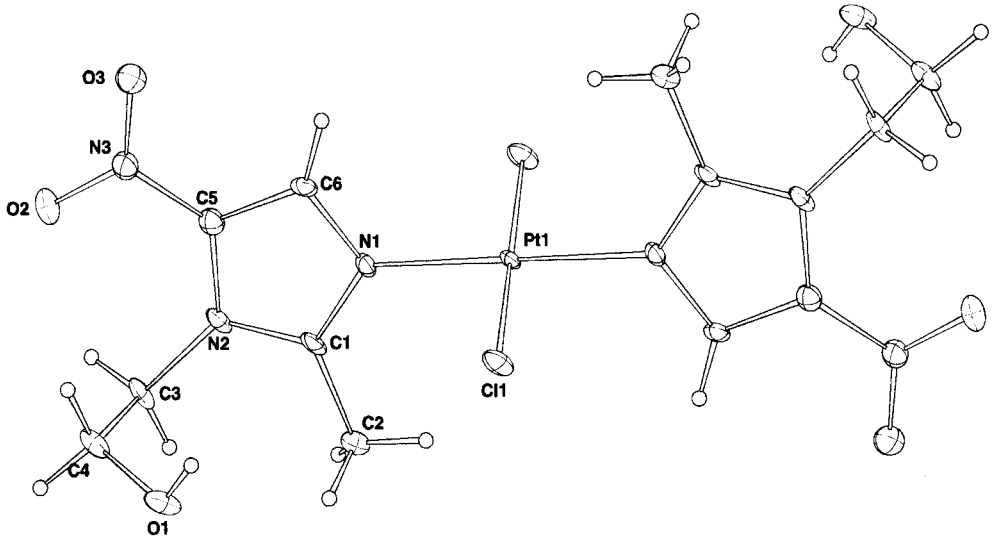

Fig. 2. *Molecular structure and atomic numbering* (arbitrary) *for complex* **2**. Ellipsoids at 30% probability.

shows all ligand substituents to be in the same orientation. The molecules are centrosymmetric and, hence, the metal ions have *trans* geometries. The central atoms Pd and Pt both have an ideal square-planar coordination geometry with M–N and M–Cl distances in accordance with reported ones [21]. The mnz ligands exhibit the expected geometries. The imidazole rings form dihedral angles of 85.98(5) and 73.73(2)° with the Cl–M–Cl plane for **1** and **2**, respectively, indicating that the rotation of the mnz ligand about the M–N bond is more restricted in the Pd complex **1**. Both

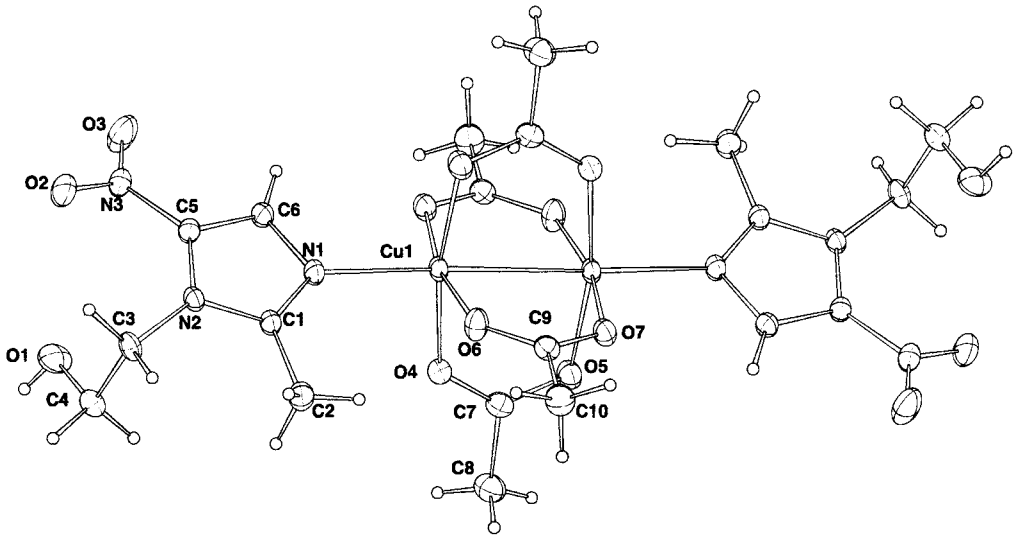

Fig. 3. *Molecular structure and atomic numbering* (arbitrary) *for complex* **3**. Ellipsoids at 30% probability.

Table 1. *Selected Bond Lengths* [Å] *and Angles* [°] *for Complexes* **1**–**3**

Complex **1**		Complex **2**			
Pd(1)–N(1)	2.0054(17)	Pt(1)–N(1)	2.006(4)		
Pd(1)–Cl(1)	2.2980(6)	Pt(1)–Cl(1)	2.292(12)		
N(1)–Pd(1)–Cl(1)	90.11(5)	N(1)–Pt(1)–Cl(1)	89.71(11)		
Complex **3**					
Cu(1)–O(4)	1.971(2)	O(4)–Cu(1)–O(5)	168.32(10)	O(5)–Cu(1)–N(1)	95.62(10)
Cu(1)–O(5)	1.971(2)	O(4)–Cu(1)–O(6)	89.87(11)	O(6)–Cu(1)–O(7)	167.66(9)
Cu(1)–O(6)	1.963(3)	O(4)–Cu(1)–O(7)	91.66(10)	O(6)–Cu(1)–N(1)	96.52(10)
Cu(1)–O(7)	1.971(2)	O(4)–Cu(1)–N(1)	96.05(10)	O(7)–Cu(1)–N(1)	95.50(10)
Cu(1)–N(1)	2.152(3)	O(5)–Cu(1)–O(6)	89.27(11)		
Cu(1)–Cu(1′)	2.6343(8)	O(5)–Cu(1)–O(7)	86.75(10)		

structures adopt the same three-dimensional supramolecular assembly, forming classic O–H···Cl and C–H···O H-bonds (O(1)–H(1)···Cl(1) and C(6)–H(6)···O(1) D···A distance = 3.166(2) and 3.189(3) Å for Pd and 3.172(4) and 3.271(6) Å for Pt, resp.).

The copper complex **3**, with the same ligand mnz in the presence of acetate forms a dimer through a Cu–Cu interaction and four bridging μ_2-acetato ligands. The structure is centrosymmetric with the Cu-centers forming a distorted octahedral coordination geometry. This distortion occurs only in the equatorial positions, which are occupied by the bridging acetato ligands whose donor groups are not sufficiently separated to span the Cu–Cu bond and form ideal octahedral geometry. The mnz ligands occupying the apical coordination sites exhibit the expected geometry, and the orientation of the imidazole ring with respect to the Cu-coordination sphere is determined by an

intramolecular interaction between the Me group at C(1) (arbitrary numbering) and an O-donor of one of the acetato bridges (D···A for C(2)–H(2C)···O(6) = 3.185(3) Å). A three-dimensional network in the crystal structure is formed by a number of intermolecular interactions (O(1)–H(1)···O(5) = 2.829(4) Å, C(3)–H(3A)··· O(7) = 3.103(6) Å, C(3)–H(3B)···O(4) = 3.428(4) Å, and C(8)–H(8a)···O(1) = 3.421(3) Å).

Biological Activity. In vitro antiamoebic activity of complexes **1**–**3** was determined with the HM1 : 1MSS strain of *E. histolytica*. The biological tests were carried out with DMSO as the solvent, in which the complexes are stable. The NMR spectra of **1**–**3** in (D_6)DMSO remained unchanged at room temperature for several days, showing no evidence of displacement of the mnz ligand by the solvent. The maximum concentration of DMSO in the test did not exceed 0.1%, at which level no inhibition of amoebal growth occurred [22]. Two-fold serial dilutions were made in either the free ligand mnz or the corresponding complexes **1**–**3** in 96-well microtiter plates and were incubated for 72 h. Cell viability was assessed, and the concentration of drug for 50% inhibition (IC_{50}) was determined by the microdilution method [23]. The effect of assayed compounds on *E. histolytica* growth is summarized in *Table 2*. Experiments were repeated thrice and the IC_{50} value was estimated to be 1.36–1.64 μM for mnz, whereas that of complex **1** was in the range of 0.085–0.148 μM. The ratio of IC_{50} of complexes **1**–**3** to mnz were 14-, 7-, and 13-fold, respectively, which indicates that complexes **1**–**3** are manyfold more active than mnz. The IC_{50} values for the Pd-, Pt-, and Cu-complex precursors were also determined establishing that these metal-complex precursors have no activity against *E. histolytica* (*Table 3*).

Table 2. IC_{50} *Values Obtained for Compounds* **1**–**3** *and Free Ligand mnz against* E. histolytica (HM1 : 1MSS)

	IC_{50} [μM][a])	Ratio
Complex **1**	0.10 ± 0.03	14.6
Complex **2**	0.20 ± 0.05	7.6
Complex **3**	0.11 ± 0.04	13.3
mnz	1.5 ± 0.2	

[a]) Mean ± 2 s.d.

Table 3. IC_{50} *Values obtained for Metal-Complex Precursors and Free Ligand mnz against* E. histolytica (HM1 : 1MSS)

	IC_{50} [μM][a])	Ratio
[$PdCl_2$[(DMSO)$_2$]	8.0 ± 1.2	0.20
[$PtCl_2$(DMSO)$_2$]	9.3 ± 1.7	0.18
[$Cu(OAc)_2$]·H_2O	7.7 ± 1.4	0.21
mnz	1.6 ± 0.3	

[a]) Mean ± 2 s.d.

In a further series of experiments, the *in vivo* antiamoebic activity of complexes **1**–**3** was assayed in male golden hamsters infected by amoeba to produce a systematic experimental amoebic hepatic abscess (EAHA). To increase the parent-strain virulence, the amoebic cultures were subjected to four liver passages and *in vitro*

culture cycles. All compounds were administered orally. Effectiveness as an anti-amoebic agent was determined as the proportion of score reduction relative to untreated controls as shown in *Fig. 4*. Treatment with 0–30 mg kg^{-1} of compounds **1**–**3** and mnz for 5 days administration did not show a clear dose-dependent effect. Treatment with 0–30 mg kg^{-1} of each compound for 10 days produced a reduction of 71–92% (**1**), 49–71% (**2**), 58–86% (**3**), and 40–58% (mnz) in the EAHA score ($P = 0.001$ compared with controls). All compounds at 10 mg kg^{-1} produced an effect quantitatively comparable with that produced by 30 mg kg^{-1} of mnz. Compound **1** at 5 mg kg^{-1} produced a reduction in EAHA that was slightly higher (4%) than the effect of 30 mg kg^{-1} of mnz.

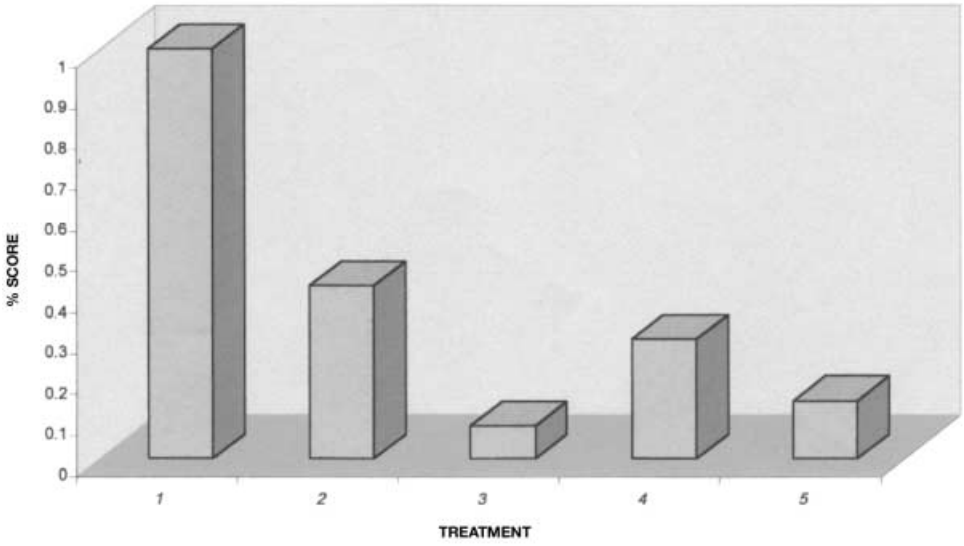

Fig. 4. *Effect of mnz and complexes* **1**–**3** *on* E. histolytica. The average scores of nontreated control and samples treated with 30 mg of compound/kg of body weight of golden hamster are shown. Scores were determined after 10 days of treatment; *1*, control; *2*, mnz; *3*, complex **1**; *4*, complex **2**; *5*, complex **3**.

These results clearly demonstrate that the (metronidazole)metal complexes **1**–**3** are potent inhibitors of the development of *E. histolytica* both *in vitro* and *in vivo* and that they are more active than the standard parent compound mnz.

Conclusions. – The metronidazole complexes of transition metals Pd, Pt, and Cu were synthesized by direct reaction of the free mnz with appropriate metal-complex precursors. *In vitro* and *in vivo* tests against *E. histolytica* showed that the incorporation of a metal ion generally produced an enhancement of the efficacy of mnz.

Experimental Part

1. *General.* All solvents were purified prior to use by standard methods. $[Cu(OAc)_2]\cdot H_2O$ (*E. Merck*), $PdCl_2$, K_2PtCl_4, and metronidazole (= mnz; *Aldrich*) were used. *trans*-$[PdCl_2(DMSO)_2]$ [20] and *cis*-$[PtCl_2(DMSO)_2]$ [20] were prepared according to the literature, and their purity was checked by UV/VIS,

IR, and ^{1}H-NMR spectra. UV/VIS Spectra: *Shimadzu UV-1601-PC*-UV-VIS spectrophotometer; λ_{max} in nm. IR Spectra: *Perkin-Elmer 1600*-FT-IR (KBr pellet in the 4000 – 400 cm^{-1} range) and *Perkin-Elmer 983* spectrometer with DS system (CsI pellet in the 600 – 150 cm^{-1} range); ν_{max} in cm^{-1}. ^{1}H-NMR Spectra: *Bruker DPX-300* spectrometer; (D_6)DMSO solns.; δ in ppm rel. to $SiMe_4$ as standard reference. Thermogravimetric analyses of the complexes were performed under N_2 with a *TG-51* thermogravimetric analyser, with the heating rate of 10°/min. Elemental analysis was performed by RSIC, Central Drug Research Institute, Lucknow, India.

2. *[SP-4-1]-Dichlorobis(2-methyl-5-nitro-1*H*-imidazole-1-ethanol-*κN^3*)palladium (*trans-[$PdCl_2$(mnz)$_2$]; **1**) *and [SP-4-1]Dichlorobis(2-methyl-5-nitro-1*H*-imidazole-1-ethanol-*κN^3*)platinum) (trans-*[$PtCl_2$(mnz)$_2$]; **2**). A soln. of mnz (684 mg, 4 mmol) in MeOH (20 ml) was added to a soln. of [$PdCl_2$(DMSO)$_2$] (665 mg, 2 mmol) or [$PtCl_2$(DMSO)$_2$] (844 mg, 2 mmol) in MeOH (20 ml) with stirring, and the mixture was refluxed at 80° for 4 h. After concentration to *ca.* 10 ml, the mixture was kept overnight in the refrigerator at 10°. The crystalline solid obtained was filtered off, washed with cold MeOH (2 × 5 ml), dried *in vacuo*, and recrystallized from ^{i}Pr-OH/MeOH 4:6.

Data of **1**: Yield 63%. Greenish-orange crystals. UV/VIS: 318. IR: 1559 (C=N), 1190 (C–O), 374 (Pd–Cl). ^{1}H-NMR (CD_3)$_2$SO): 3.06 (*s*, 2 Me); 3.71 (*q*, 2 CH_2); 4.44 (*t*, 2 CH_2); 5.13 (*t*, 2 OH); 8.29 (*s*, 2 arom. H). Anal. calc. for $C_{12}H_{18}Cl_2N_6O_6$ Pd: C 27.74, H 3.46, N 16.18; found: C 27.96, H 3.63, N 16.04.

Data of **2**: Yield 57%. Yellow-orange crystals. UV/VIS: 310. IR: 1558 (C=N), 1187 (C–O), 361 (Pt–Cl). ^{1}H-NMR (CD_3)$_2$SO): 3.0 (*s*, 2 Me); 3.71 (*q*, 2 CH_2); 4.49 (*t*, 2 CH_2); 5.16 (*t*, 2 OH); 8.24 (*s*, 2 arom. H). Anal. calc. for $C_{12}H_{18}Cl_2N_6O_6$ Pt: C 23.68, H 2.96, N 13.71; found: C 23.80, H 3.15, N 13.47.

3. *Tetrakis[μ-(acetato-*κO:κO′*)bis(2-methyl-5-nitro-1*H*-imidazole-1-ethanol-*κN^3*)dicopper* ([Cu_2(μ-OAc)$_4$(mnz)$_2$]; **3**). A soln. of [Cu(OAc)$_2$]·H_2O (400 mg, 2 mmol) in MeOH (50 ml) was added with stirring to mnz (342 mg, 2 mmol) in MeOH (15 ml). The mixture was stirred at r.t. for 48 h. On concentration of the mixture, greenish-blue crystals precipitated, which were filtered off, washed with cold MeOH, dried *in vacuo*, and recrystallized from MeOH/DMSO 9:1: **3** (63%). Greenish-blue crystals. UV/VIS: 317. IR: 1548 (C=N), 1189 (C–O). Anal. calc. for $C_{20}H_{32}Cu_2N_6O_{14}$: C 34.09, H 4.26, N 11.93; found: C 34.26, H 4.46, N 11.90.

4. *Crystal Structures of Complexes* **1**–**3**. Crystals of **1**–**3** were mounted on glass fibers by means of the oil-drop technique, and intensity data were collected at 150 K. A summary of crystal data, data collection strategy, and mode-refinement parameters are given in *Table 4*. Data were measured on a *Nonius Kappa CCD* area-detector diffractometer with a rotating anode molybdenum (λ(Mo-K_α) 0.71073 Å) target, controlled by DENZO and COLLECT [24] software. The structures were solved *via* direct methods and then subjected to full-matrix least-squares refinement on F_o^2 with the SHELX-97 [25] suite of programs.

For all structures, non-H-atoms were made anisotropic, with H-atoms in calculated positions (C–H = 0.96 Å, with U_{iso} tied to U_{eq} of the parent atoms) for structures **2** and **3**, whilst H-atom parameters were freely refined for structure **1**. Absorption corrections were performed by means of comparison of symmetry-related and equivalent reflections with the method employed in SORTAV [26]; however, due to the large size of the crystal of **3**, some large residual-density peaks remained around the Cu center. Moreover, the alcohol group at C(4) (arbitrary numbering) of complex **3** was disordered over two sites (in an 80:20 ratio) with a fractionally occupied H-atom completing the site requirements. Full crystallographic data for **1**–**3** have been submitted to *CCDC* (deposition Nos. 155790 – 155792).

5. *Organism Culture and* in vitro *Testing against* E. histolytica. Preliminary experiments were carried out to determine the antiamoebic activities of the *in vitro* culture against the HM1:1MSS strain of *E. histolytica* as previously described [23]. The *E. histolytica* strain HM1:1MSS was cultured in tubes (15 ml) with *Diamond TYIS-33* medium [27]. All the compounds were dissolved in DMSO (40 μl) [22][28], followed by adding enough culture medium to obtain a concentration of 1 mg/ml. Stock solutions of the compounds were prepared freshly before use at a concentration of 0.1 mg/ml. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (*Costar*). Each test included mnz as a standard amoebicidal drug, with control wells (culture medium plus amoebae) and a blank (culture medium only). The amoebae suspension was prepared from a confluent culture by pouring off the medium at 37° and adding 5 ml of fresh medium, chilling the culture tube on ice to detach the organisms from the side of the flask. The number of amoeba/ml was estimated with a heamocytometer, by means of trypan-blue exclusion to confirm the viability. The suspension was diluted to 10^5 organism/ml by adding fresh medium, and 170 μl of this suspension was added to the test and control wells in the plate so that the wells were completely filled (total volume, 340 μl). An inoculum of $1.7 \cdot 10^4$ organisms/well was chosen so that confluent, but not excessive growth, took place in control wells. Plates were sealed and gassed for 10 min with N_2 before incubation at 37° for 72 h. After incubation, the growth of amoebae in the plate was checked with a low-power microscope. By inverting the plate, the culture medium was removed with gentle shaking and then immediately washed with 0.9% NaCl soln. at 37°. This procedure was completed quickly, and the plate was not

Table 4. *Crystal Data and Structure-Refinement Details for Compounds* **1**–**3**

	1	**2**	**3**
Empirical formula	$C_{12}H_{18}Cl_2N_6O_6Pd$	$C_{12}H_{18}Cl_2N_6O_6Pt$	$C_{20}H_{30}CuN_6O_{14}$
M_r	519.62	608.31	352.79
Crystal size [mm]	$0.22 \times 0.2 \times 0.05$	$0.2 \times 0.2 \times 0.2$	$0.33 \times 0.3 \times 0.25$
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$	$P2_1/c$
a [Å]	7.1328(14)	6.9169(14)	9.1663(18)
b [Å]	20.699(4)	21.853(4)	19.129(4)
c [Å]	7.1455(14)	6.7218(13)	8.9446(18)
β [°]	116.17(3)	110.79(3)	116.44(3)
Volume [Å^3]	946.8(3)	949.9(3)	1404.3(5)
Z	2	2	2
Density (calc.) [Mg/m^3]	1.823	2.127	1.669
Absorption coefficient [mm^{-1}]	1.305	7.710	1.592
$F(000)$	520	584	724
θ_{max} °	27.51	27.49	27.45
Reflections collected	10091	4908	10714
Independent reflections	2165	2123	3137
R (int)	0.0487	0.0439	0.0748
Transmission factors	0.9376, 0.7578	0.3077, 0.3077	0.6916, 0.6256
Final R indices	$R_1 = 0.0229$	$R_1 = 0.0433$	$R_1 = 0.0555$
$F^2 > 2\sigma F^2$	$wR_2 = 0.0538$	$wR_2 = 0.1192$	$wR_2 = 0.1507$
	(1872 reflections)	(1832 reflections)	(2718 reflections)
$\Delta\rho$ max, min [eÅ^{-3}]	0.786, −0.716	3.764, −3.210	2.229, −0.856

allowed to cool to prevent the detachment of amoebae. It was allowed to dry at r.t. After drying, amoebae were fixed with chilled MeOH by keeping it in an ice bath for 15 min, dried, and stained with 0.5% aq. eosin soln. for 15 min. The stained plate was washed once with tap water, and then twice with distilled water and allowed to dry. A 200-µl portion of 0.1N NaOH was added to each well to dissolve the protein and release the dye. The optical density of the resulting soln. in each well was determined at 490 nm with a microplate reader. The % inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear-regression analysis was used to determine the best-fitting straight line from which the IC_{50} value was found.

6. In vivo *Antiamoebic Activity. Animals.* Male golden hamsters (*Mesocricetus auratus*), aged one month and weighing 50–70 g were used. During this study, the animals were maintained in polypropylene cages, with food and water *ad libitum. Amoebae. E. histolytica*, strain HM1:1MSS, trophozoites were used in all the experiments. Amoeba cultures were maintained under axenic conditions in TYIS-33 medium with a subcultivation frequency of 4 days. These amoebae were used to produce EAHA in hamsters. To increase the parent-strain virulence, the amoebic cultures were subjected to four liver passages and *in vitro* culture cycles [29].

Experimental Amoebic Hepatic Abscess (EAHA) Production. The animals were anesthetized with 6.3 mg i.p./100 g of body weight of sodium pentobarbital (*Anestesal*®, from *Smith Kline & French*, Mexico D.F.). A laparatomy was practiced on each animal under aseptic conditions. The left hepatic lobe was exposed and inoculated with $5 \cdot 10^5$ trophozoites suspended in 0.1 ml of fresh TYI-33 medium (without serum). The inoculation was done with a disposable tuberculin syringe, which was armed with a 25-gauge hypodermic needle. The incision was closed with a continuous suture. The animals were maintained with food and water *ad libitum* in their cages during the following seven days. Afterwards, another laparatomy was practiced to verify the EAHA development in each animal. The treatment was started 24 h after exploratory laparatomy.

Drugs. Compounds **1**–**3** or mnz were dissolved in DMSO. The concentration of each of the above drugs was properly adjusted with buffer, to be administered at the required doses, in 0.5-ml volumes. These were administered orally with an intragastric canula.

Treatment Schemes. The animals were divided into ten groups (I–X) for each **1**–**3** and mnz. Groups I and X were untreated controls, groups *II*, *III*, *IV*, and *V* were treated with 5, 10, 20, and 30 mg kg^{-1} of each compound

for five days, and groups *VI*, *VII*, *VIII*, and *IX* were treated with 5, 10, 20, and 30 mg kg^{-1} of each compound for ten days. Twenty-four hours after the last dose of drug, the animals were killed with an i.p. injection of sodium pentobarbitone, and the EAHA in each animal was scored according to the following criteria: 0, liver showing the inoculation wound only or EAHA < 1 mm in diameter; 1, 1–5 mm diameter lesions; 2, 5–10 mm diameter lesions; 3, 10–15 mm diameter lesions, with minimal increase in lobe volume; 4, 15–20 mm diameter lesions with multiple satellite lesions of 1–5 mm diameter; 5, 20–30 mm diameter lesions with EAHA equivalent to 75% or more of the lobe volume, showing multiple satellite lesions and noticeable increase in the lobe volume. Effectiveness as an antiamoebic agent was determined as the proportion of score reduction by the drug relative to untreated controls. The significance of the above comparisons was estimated by applying the *Kruskal-Wallis*'s one-way ANOVA nonparametric test, with the help of the SPSS statistical package program installed on a personal computer (SPSS, Inc. Ver 5.0).

N. Bharti acknowledges the *Council of Scientific and Industrial Research*, New Delhi, India, for a *Senior Research Fellowship*. The authors are thankful to Prof. *Alok Bhattacharya* and Dr. *Sudha Bhattacharya*, School of Life Sciences and School of Environmental Sciences, respectively, Jawaharlal Nehru University, New Delhi, for providing laboratory facilities for the determination of biological activities.

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Received March 8, 2002